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Alternative GC–MS approaches in the analysis of substituted pyrazines and other volatile aromatic compounds formed during Maillard reaction in potato chips

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ABSTRACT

Several methods have been developed for the analysis of substituted pyrazines and related substances in potato chips. Following separation/detection approaches (all employing head-space solid phase microextraction, HS-SPME, for volatiles sampling), have been critically assessed in our study: (i) gas chromatography–ion trap mass spectrometry (GC–ITMS), (ii) gas chromatography–time-of-flight mass spectrometry (GC–TOFMS); (iii) comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOFMS). Although in none of the tested systems full chromatographic resolution of some isomeric pairs could be achieved, the use of GC × GC–TOFMS offered the best solution, mainly because of distinctly lower limits of quantification (LOQs) for all of 13 target alkylpyrazines. In addition to good performance characteristics, a non-target screening and tentative identification of altogether 46 *N*-containing heterocyclic compounds (pyrazines, pyrrols, pyridines, pyrrolidinones, and tetrahydropyridines) was also enabled.

1. Introduction

A wide range of reactions occurs during thermal processing of food commodities. Depending on various factors, including temperature, time, pH value, etc., many products are formed from precursors present in a particular raw material. On this account, characteristic flavour and colour originate, also the texture and nutritional value of the product are influenced by heat-treatment. Maillard reaction between carbonyl and amino compounds, taking place at elevated temperatures (>80 °C), is responsible for many of these changes [1]. Besides of health promoting compounds such as antioxidants, antinutritive and even toxic substances can be formed via Maillard reaction [2–6]. At the beginning of this century, the presence of acrylamide, compound classified by the International Agency for Research on Cancer (IARC) as "probable human carcinogen" (2B) [7], was documented in various heat processed cereal and potato-based foodstuffs [8,9].

Numerous studies aimed at elucidation of acrylamide formation showed that a reaction between reducing sugars and asparagine is the main pathway yielding this processing contaminant [10]. Potato chips were found to be a main dietary source of acrylamide, therefore the need to control its level in this commodity is important. It should be noted, that analysis of acrylamide (LC–MS/MS methods is most often employed) in complex matrices such as food is rather cost and labour demanding, an intensive purification of crude extract is necessary to obtain low detection limits [11].

To overcome these difficulties, the possibility to implement a simple, high throughput procedure enabling to monitor some volatile markers of acrylamide formation, was searched. In preliminary model experiments, the release of alkylpyrazines, typical volatile products of Maillard reaction, was shown to correlate with acrylamide formation [12]. On this account, comprehensive research focused on the analysis of volatile fraction of potato chips can serve as an alternative strategy of acrylamide control.

A wide range of approaches has been used for this purpose. The isolation of volatiles, prior to gas chromatography employing either a flame ionisation detection (FID) or a mass spectrometric (MS) detection, can be carried out as follows: (i) extraction by means of Likens-Nickerson [13,14] or Soxhlet apparatus [15]; (ii) high-vacuum distillation supported by the solvent-assisted flavour evaporation (SAFE) technique [16]; (iii) dynamic or static head-space technique [3,14]; (iv) purge-and-trap technique [15].

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Surprisingly, none of the studies concerned with potato chips volatiles employed solid phase microextraction (SPME) technique for extraction/pre-concentration of these compounds. This inexpensive, solvent-free sampling technique enables convenient isolation of a wide range of low molecular weight analytes by their extraction from sample head-space and their concentration on the fibre coating [17].

In our study, a SPME method for the head-space sampling of alkylpyrazines and other volatile compounds emitted from potato chips, was optimised and validated. GC coupled to an ion trap MS detector (GC–ITMS) and also comprehensive two-dimensional gas chromatography coupled to a time-of-flight MS detector (GC × GC–TOFMS) were used for the identification of the target analytes. The comparison of performance characteristics of the above approaches as well as discussion of the benefits of the GC × GC–TOFMS are presented in this paper.

2. Experimental

2.1. Chemicals, materials, and standard solutions

Alkylpyrazines (2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, a mixture of 2-ethyl-5-methylpyrazine and 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, 2,3-diethylpyrazine, a mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-butyl-3-methylpyrazine and 2-*n*-propylpyrazine) were purchased from Pyrazine Specialties, INC. (Atlanta, USA). Methanol was supplied by Merck (Darmstadt, Germany) and purified water was prepared using a Milli Q RG apparatus (Millipore, USA). The stock solutions of individual pyrazines (1–2 mg mL^{-1}) were prepared by dissolving these compounds in methanol. The stock solutions were stored at 4 °C. A mixed standard of all pyrazines in methanol (47–104 μg mL^{-1}) was prepared from the individual standards solutions. The calibration standards were prepared at six concentration levels by dilution of the mixed stock solution in methanol (1:2, 1:5, 1:10, 1:50, 1:100, and 1:500; v/v). The calibration curve was measured in the presence of the matrix (potato chips). For this purpose, 5 μL of the standard solution were added to 1.5 g of moderately fried (5 min/160 °C) potato chips, tested beforehand for the absence of pyrazines.

2.2. Sample preparation and SPME conditions

A mixed standard of pyrazines for GC optimization was prepared by adding 5 μL of the mixed standard solution in methanol into a 10 mL head-space vial containing 5 mL of distilled water.

2.2.1. Commercially prepared samples

A portion of 1000 g of potato slices was fried in the deep fryer (110 L of refined rape oil, the temperature of oil bath before start of frying was ca. 162 °C). Approximately 50 g of sample was taken after 2.5 and 6 min of frying. The ground sample was stored in a closed vessel.

2.2.2. Lab-fried samples

A portion of 400 g of potato slices was fried in the deep fryer (4 L of rape and sunflower oil mixture, the temperature of oil bath before start of frying was ca. 160 °C). Approximately 50 g of sample was taken after 5 and 20 min of frying. The ground sample was stored in a closed vessel.

A portion of 1.50 g of ground chips was placed into a 10 mL head-space vial and capped.

Ten milliliters head-space vials (Sigma–Aldrich, USA) were cleaned using sonication in water with detergent, purified water and acetone (20 min each). Clean vials were dried at 220 °C for 4 h, and then stored covered with an aluminium foil.

Four different fibres – 65 μm carbowax/divinylbenzene (CW/DVB), 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), and 30/50 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) – purchased from Supelco (USA), were tested for the head-space sampling of volatile compounds from potato chips.

New fibres were conditioned for 4 h in a GC injector according to the manufacturer's recommendations. Short conditioning (30 min/250 °C) and a blank run of the fibre were carried out daily before its use for sampling.

Incubation of samples was performed for 5 min at 30 °C, followed by 60 min (GC–ITMS) or 15 min (GC–TOFMS, GC × GC–TOFMS) sorption using an SPME fibre, and 5 min (GC–ITMS) or 2 min (GC–TOFMS, GC × GC–TOFMS) desorption at 250 °C.

2.3. Instrumentation

2.3.1. GC–ITMS system

A gas chromatograph Trace GC 2000 (Thermo Quest, USA) equipped with an ion trap mass spectrometric detector POLARIS Q (Finnigan, USA) was used for identification and quantification of the analytes. Automated HS-SPME was performed using a CombiPal multipurpose sampler (CTC Analytics, Switzerland).

2.3.1.1. GC–ITMS conditions. An HP-VOC fused-silica capillary column 60 m × 0.2 mm, 1.1 μm (Agilent, USA) and helium as a carrier gas (1 mL min^{-1}) were used.

Set-up A (initial experiments): The column was held at 40 °C for 5 min, the oven temperature was then programmed up to 120 °C at a rate of 3 °C min^{-1}, then up to 260 °C at a rate of 6 °C min^{-1} (GC run time was 55 min).

Set-up B (follow-up experiments): The column was held at 40 °C for 5 min, the oven temperature was then programmed up to 120 °C at a rate of 15 °C min^{-1}, then up to 150 °C at a rate of 1.5 °C min^{-1} and finally up to 260 °C at 10 °C min^{-1} (GC run time was 41 min).

The injector was operated in splitless mode using 5 min desorption time. The temperature of the injection port was set at 250 °C. The GC transfer line was maintained at 275 °C, the temperature of ion source was held at 200 °C. The filament emission current was set at 250 μA. Mass spectrometer operating in the electron ionisation (EI) mode was set to the segment scan detection mode using following segments: *m*/*z* 25–80, 81–110, 111–155, 156–200, 201–300, and 301–450.

Data collection and processing was performed by means of the software XCALIBUR, version 1.2.2 (Finnigan, USA).

2.3.2. GC × GC–TOFMS system

GC × GC–TOFMS instrument Pegasus 4D consisted of an Agilent 6890N gas chromatograph with a split–splitless injector and a time-of-flight mass spectrometer Pegasus III with 10 mL min^{-1} pumping capacity (LECO Corp., St. Joseph, USA). Inside the GC oven a dual-stage jet modulator and a secondary oven were mounted (LECO). Resistively heated air was used as a medium for hot jets, while cold jets were supplied by gaseous nitrogen, cooled by liquid nitrogen.

Automated HS-SPME of volatiles was performed using an MPS2 autosampler (Gerstel, Germany).

2.3.2.1. 1D-GC–TOFMS conditions. An HP-VOC fused-silica capillary column 60 m × 0.2 mm, 1.1 μm (Agilent, USA) and helium as a carrier gas (1.5 mL min^{-1}) were used. The column was held at 40 °C for 2 min, the oven temperature was then programmed up to 130 °C at a rate of 20 °C min^{-1}, then up to 170 °C at a rate of 1.5 °C min^{-1} and finally up to 260 °C at 20 °C min^{-1} (GC run time was 38 min).

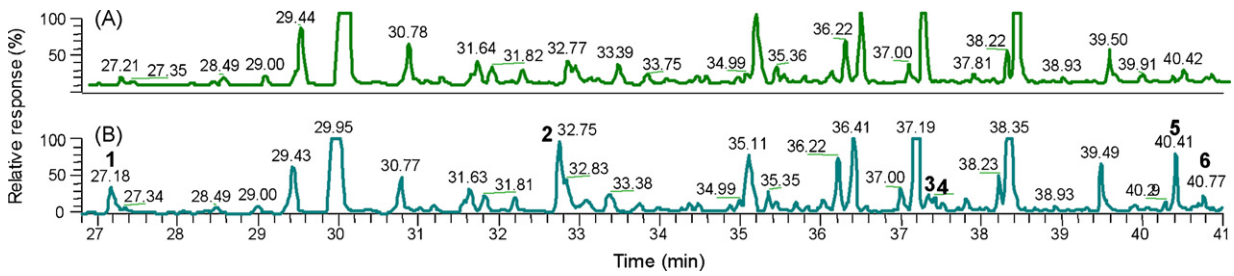

Fig. 1. Aroma profile of chips fried at approximately 162 °C for (A) 2.5 min and (B) 6 min. The GC–ITMS (set-up A) used for sample separation. Marked compounds: (1) methylpyrazine; (2) 2,5-dimethylpyrazine and 2,6-dimethylpyrazine; (3) 2-ethyl-6-methylpyrazine; (4) trimethylpyrazine; (5) 2,6-diethylpyrazine; (6) 2-*n*-propylpyrazine.

The injector was operated in splitless mode using 2 min desorption time. The temperature of injection port was set at 250 °C. GC transfer line was maintained at 250 °C. The mass spectrometer was operated in the EI mode with a source temperature of 220 °C. An acquisition rate of 5 spectra s^{-1} and stored mass range m/z 35–470 were used.

2.3.2.2. GC × GC–TOFMS conditions. For GC × GC, a Supelcowax 10 2.5 m × 0.1 mm, 0.1 μm (Supelco, USA) column was used in the second dimension. The secondary oven was held at 45 °C for 2 min, the oven temperature was then programmed up to 135 °C at a rate of 20 °C min^{-1}, then up to 175 °C at a rate of 1.5 °C min^{-1} and finally up to 265 °C at 20 °C min^{-1} (GC run time was 38 min). The modulation time 5 s (hot pulse 1 s) and modulation temperature offset 15 °C were applied.

Other GC parameters including injection conditions and the main oven program were the same as in the 1D-GC–TOFMS analysis. MS acquisition rate was 180 spectra s^{-1} to obtain sufficient number of points per chromatographic peaks. Other MS parameters were the same as for the 1D-GC–TOFMS analysis.

3. Results and discussion

As mentioned in Section 1, this study was conducted within the project concerned with monitoring of acrylamide formation during the potato chips frying. Having in mind laborious and time demanding acrylamide analysis by LC–MS/MS, we decided to test the possibility to apply an alternative approach, which employs a simpler SPME–GC–MS procedure for monitoring of pyrazines, possible volatile markers of this processing contaminant. Both, the optimisation of the sample preparation and the final determination of the analytes, are discussed in the following paragraphs.

3.1. SPME sampling

In the first phase of the SPME experiments, four different fibres were tested. Since the profile of head-space volatiles is changing during potato chips preparation, two samples varying in frying time (2.5 and 6 min) were examined. With regard to a high extraction efficiency of the whole range of the volatiles released from the potato chips samples, the divinylbenzene/carboxene/polydimethylsiloxane (30/50 μm DVB/CAR/PDMS) fibre was selected for further experiments. In Fig. 1, the aroma profiles of the not enough fried chips (fried for 2.5 min) and of the final product (fried for 6 min) are shown. Based on a thorough assessment of these chromatograms, the most significant compounds found to be associated with Maillard reaction, were pyrazines in the later sample. In both samples, various carbonyls and other breakdown products originated in oil frying bath, were the most abundant compounds present in chips head-space.

To get information on the possible relationship between the sample surface and the amount of emitted volatiles, and also to identify potential losses of the most volatile compounds, two ways of the potato chips treatment prior to SPME sampling, i.e. grinding and milling, were tested. Since no differences in pyrazine profiles and intensities were observed, the later (simpler) procedure was used in follow-up experiments.

In the next step focused on the SPME optimisation, several sorption times were tested. As shown in Fig. 2, the peak areas of pyrazines increased linearly all over the tested sampling range (5–60 min), the equilibrium was not achieved even at the longest sorption time (60 min). As a compromise between the requirement for quick analyses and sufficient method sensitivity, the 60 min sampling was selected for subsequent experiments employing GC–ITMS instrumentation.

The sorption temperature is another important parameter influencing the whole SPME procedure. Although, within a certain range, increased temperature resulted in enhanced SPME sensitivity, which was caused by increased transfer of volatiles into the sample head-space, it was not possible to utilise this phenomena because additional formation of pyrazines could be induced at higher temperatures. On this account, the sorption temperature of 30 °C was used throughout all the following experiments.

3.2. GC–ITMS analysis

Since the original aim of our study was to find possible volatile markers of acrylamide formation during potato chips frying, an HP-VOC fused-silica capillary column seemed us to be suitable for this purpose. In Fig. 3, a chromatogram of volatiles obtained under optimised SPME conditions is presented.

Unfortunately, using this GC capillary column, neither the chromatographic nor spectral resolution of the following isomeric pyrazine pairs (i) peak 2 + 3 (2,5(6)-dimethylpyrazine), (ii) peak 4(5) + 6 (2-ethyl-5(6)-methylpyrazine with 2,3,5-trimethylpyrazine) and (iii) peak 8(9) + 10 (2-ethyl-3,5(6)-dimethylpyrazine with 2,3-diethylpyrazine) was achieved.

Another difficulty we encountered in this GC arrangement was the identification of the elution order within two pairs

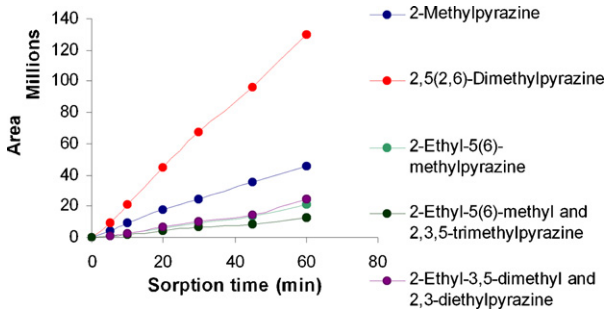

Fig. 2. Optimisation of HS-SPME sorption time, 30/50 μm DVB/CAR/PDMS fibre, sorption temperature 30 °C.

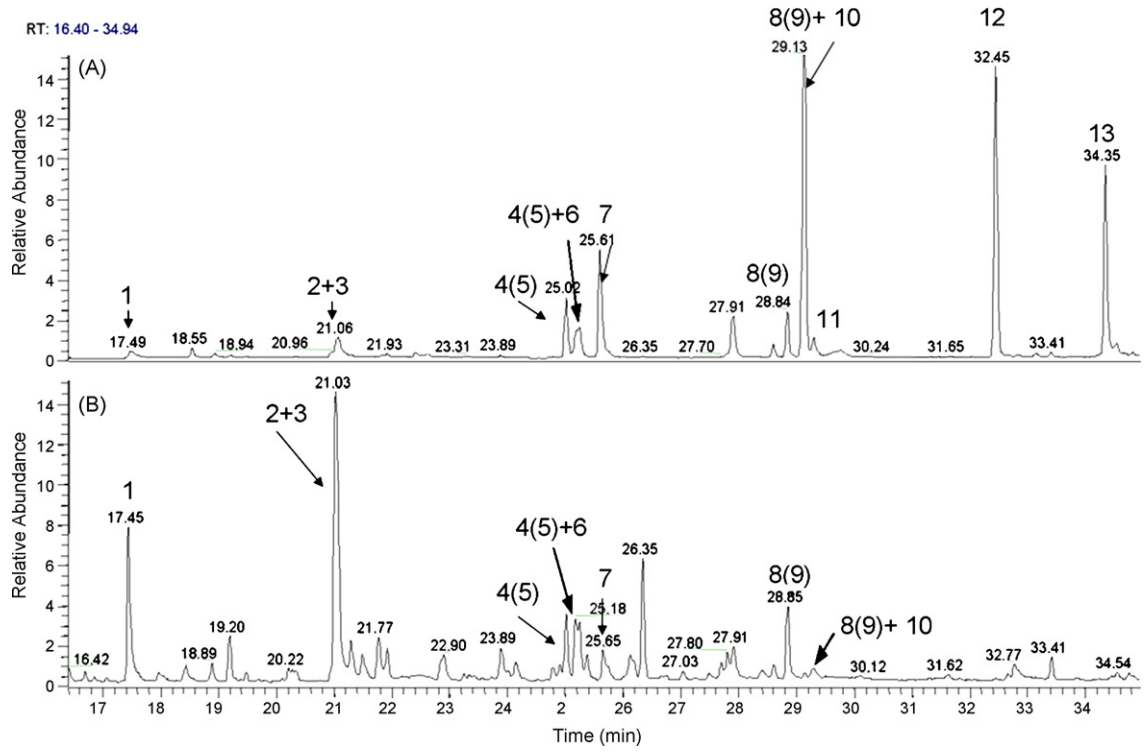

Fig. 3. Total ion current chromatograms of (A) mixed standard of pyrazines and (B) potato chips sample under the experimental conditions of GC–ITMS (set-up B). Denoted analytes: (1) 2-methylpyrazine; (2) 2,5-dimethylpyrazine; (3) 2,6-dimethylpyrazine; (4) 2-ethyl-6-methylpyrazine; (5) 2-ethyl-5-methylpyrazine; (6) 2,3,5-trimethylpyrazine; (7) 2-*n*-propylpyrazine; (8) 2-ethyl-3,5-dimethylyrazine; (9) 2-ethyl-3,6-dimethylpyrazine; (10) 2,3-diethylpyrazine; (11) 2,3,5,6-tetramethylpyrazine; (12) 2,3-diethyl-5-methylpyrazine; (13) 2-butyl-3-methylpyrazine. (*Note:* The number in parenthesis is an alternative elution order for isomeric pair, which is unknown.)

of isomeric pyrazines: 4(5) (2-ethyl-5(6)-methylpyrazine) and 8(9) (2-ethyl-3,(5)6-dimethylpyrazine). These analytes were commercially available only as the mixtures of standards and the corresponding retention indexes on HP-VOC column, which could help to differentiate between them, were not found in literature.

3.3. 1D-GC and GC × GC–TOFMS analysis

As an alternative to separation system employing a conventional MS detector such as ion trap, we also tested hyphenation of GC to the TOFMS. Although ITMS can (alike TOFMS) provide a full spectral information throughout the GC run, the TOFMS technique may provide higher detection sensitivity typically at pg level [18].

Contrary to the scanning mass analysers, no spectral skewing occurs across the GC peak using the TOFMS detection, hence most of the overlapping peaks can be deconvoluted using a suitable software, ChromaTOF in this particular case. Fig. 4 shows a chromatogram obtained by SPME–GC–TOFMS analysis of the chips.

Automated peak finding, background subtraction, and the deconvolution option were employed as powerful tools for the processing of GC–TOFMS chromatograms.

Spectral resolution of two critical pairs 2,5-dimethylpyrazine (2) + 2,6-dimethylpyrazine (3) and 2-ethyl-5(6)-methylpyrazine (4(5)) + 2,3,5-trimethylpyrazine (6) was achieved, clean mass spectra with high library match factors (e.g. similarity 932 and 908 for 2,5-dimethylpyrazine and 2,6-dimethylpyrazine, respectively) were available for each of the co-eluted pyrazines. Although their fragmentation patterns were fairly similar, small differences in the low intensity ions (*m*/*z* 67 and 80 in the case of 2,5-dimethylpyrazine + 2,6-dimethylpyrazine, *m*/*z* 121 and 42 in the case of 2-ethyl-5(6)-methylpyrazine + 2,3,5-trimethylpyrazine) were sufficient enough for their spectral resolution. Unfortunately, the third critical pair consisting of 2-ethyl-3,5(6)-dimethylpyrazine (8(9)) + 2,3-diethylpyrazine (10) could not be resolved due to the absolutely identical retention times and peak shapes.

Altogether, the presence of all the 13 alkylpyrazines, for which analytical standards were available, was documented in chips sample, three more, minor pyrazines (2,3,5,6-tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine and 2-butyl-3-methylpyrazine) were detected thanks to better sensitivity of TOFMS compare to ITMS.

However, the unambiguous identification of pyrazines within the isomeric pair 2-ethyl-6-methylpyrazine (4) and 2-ethyl-5-methylpyrazine (5), as well as the pair 2-ethyl-3,5-dimethylpyrazine (8) and 2-ethyl-3,6-dimethylpyrazine (9), was, for the same reason as in the case of GC–ITMS determination, impossible using the GC–TOFMS system.

Because of the problems with resolution described above, we attempted to improve the chromatographic separation by introducing a comprehensive two-dimensional GC coupled to TOFMS (GC × GC–TOFMS). The first dimension narrow bore HP-VOC column (the same as that used in previous experiments) was coupled via a thermal modulator with a short microbore capillary coated by Supelcowax 10 (polyethylene glycol phase) providing a different selectivity in the second dimension. Under this experimental set-up, each peak eluted from the HP-VOC column had to be modulated two to three times to preserve the first dimension separation. Very fast elution of sample components in the second column enabled separation to be completed before the next modulation started. In this way, each compound in the volatiles mixture was a subject of two independent separation mechanisms [19,20], what largely enhanced the overall resolution power.

In Fig. 5, a contour plot obtained by the analysis of alkylpyrazines standard mixture (Fig. 5A) and potato chips volatiles (Fig. 5B) is shown. Rather surprisingly, neither chromatographic nor spectral resolution was achieved (contrary to 1D-GC–TOFMS system) for pair consisting of 2,5-dimethylpyrazine (2) and 2,6-dimethylpyrazine (3). A recombination during the modulation process probably

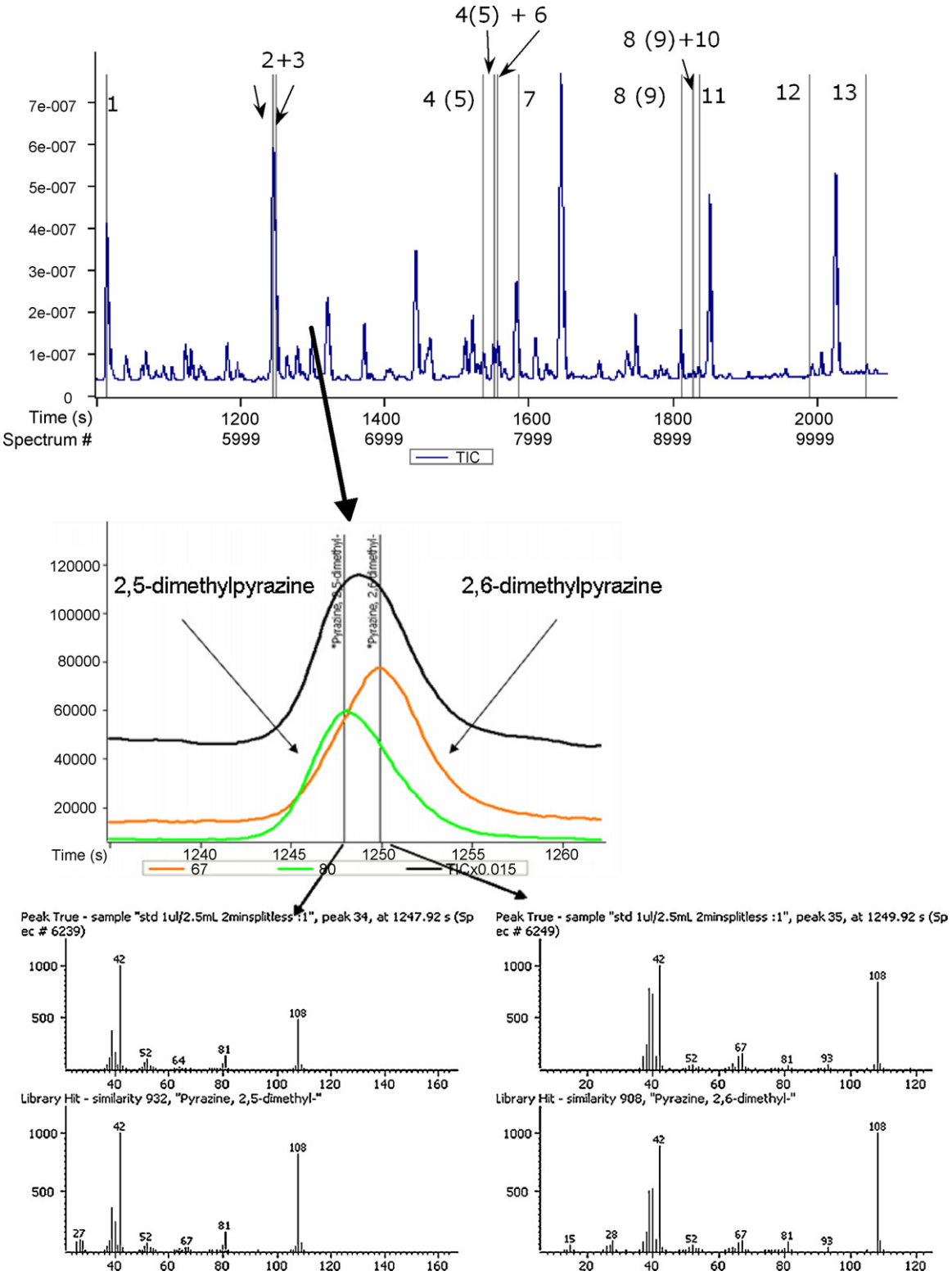

Fig. 4. 1D-GC–TOFMS analysis of potato chips sample: total ion current and deconvolution of 2,5-dimethylpyrazine and 2,6-dimethylpyrazine. For identification of compounds see caption of Fig. 3.

occurred. It should be noted that the setting of a (relatively long) 5-s modulation period avoided so-called "wrap-around", i.e. elution of more retained compounds at retention times higher than the modulation period. Similarly, no improvement was obtained for the pairs 2-ethyl-5(6)-methylpyrazine (4(5)) + 2,3,5-trimethylpyrazine (6) and 2-ethyl-3,5(6)-dimethylpyrazine (8(9)) + 2,3-diethylpyrazine (10).

Although the expectation that GC × GC system would resolve co-elution problems was not fulfilled, this set-up enabled a better detectability of pyrazines. Thanks to compression of the peaks in a modulator and separation of the target analytes from the other sample components (based on the differences in their polarity), the signal-to-noise ratio increased approx. by a factor of 4. Improved detection limits allowed reduction of the SPME sorption time to

only 15 min (instead of previously used 60 min), as a consequence the sample throughput increased significantly.

3.4. Non-target analysis of heterocyclic nitrogen-containing compounds

In addition to analysis of the target alkylpyrazines, also other (semi)volatile nitrogen-containing heterocyclic compounds originated via Maillard reaction during potato chips frying were searched. For this purpose, both the data from 1D-GC–TOFMS and GC × GC–TOFMS analysis were examined by ChromaTOF software using a threshold (S/N) setting of 50. The automatically generated peak table composed of compounds exceeding this value at each single ion trace in a mass range of m/z 35–470, and identified after deconvolution on the basis of measured mass spectra comparison with NIST 2002 library, was carefully examined. The list of altogether 46 non-target compounds representing 5 structure groups (19 pyrazines, 11 pyrrols, 10 pyridines, 4 pyrrolidinones, and 2 tetrahydropyridines) tentatively identified in this way is given in Table 1. Substances marked in this table by a superscript a were identified in both systems (1D-GC–TOFMS and GC × GC–TOFMS), those marked by a superscript b could be identified only under conditions of GC × GC, i.e. when the combination of two separation principles (based on volatility and polarity) enabled improved spectral quality (most of co-elutions with matrix components were avoided). An example documenting the benefits resulting from this approach is illustrated in Fig. 6, in which 2-ethenyl-5-methylpyrazine was fully separated from d-limonene. Due to the co-elution with this abundant matrix component, identification of the above pyrazine was not possible under the conditions of conventional1D-GC run.

In spite of unavailability of authentic standards needed for the identity confirmation, the unique features of the mass spectra of *N*-heterocyclic compounds limited the misidentifications to only the incorrect assignment of the isomeric forms.

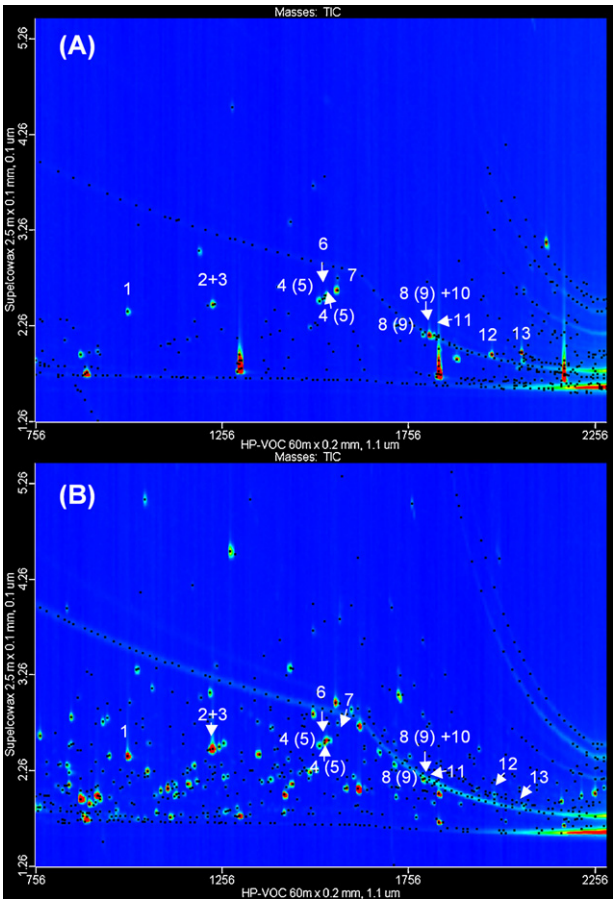

Fig. 5. GC × GC–TOFMS analysis of (A) mixed standard of alkylpyrazines and (B) potato chips sample. For peak numbering refer to Fig. 3.

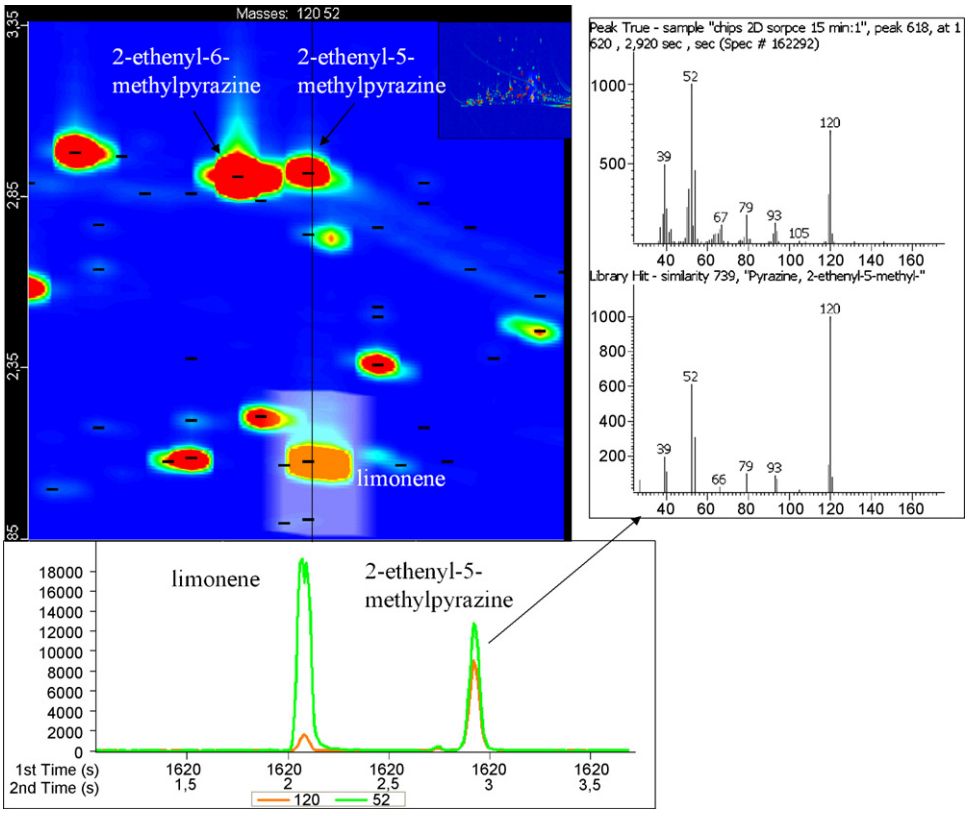

Fig. 6. Separation of 2-ethenyl-5-methylpyrazine and limonene using GC × GC–TOFMS.

Table 1
The list of nitrogen-containing compounds tentatively identified in potato chips head-space by GC(×GC)–TOFMS.

$^{1D}t_R$ (s), $^{2D}t_R$ (s)	Peak numbering	Name	Quant mass[c]	Formula
822, 2.340		Pyrazine[a,d]	80	C4H4N2
840, 3.960		Pyrrole[a,d]	67	C4H5N
846, 2.250		Pyridine[b,d]	79	C5H5N
972, 2.200		1-Ethyl-1*H*-pyrrole[b]	80	C6H9N
984, 2.280		2-Methylpyridine[a]	93	C6H7N
1002, 2.420	1	2-Methylpyrazine[a]	94	C5H6N2
1014, 3.990		2-Methyl-1*H*-pyrrole[b,d]	80	C5H7N
1038, 4.130		3-Methyl-1*H*-pyrrole[b]	80	C5H7N
1134, 2.470		Pyridine, 3-methyl-[b]	93	C6H7N
1140, 2.280		Pyridine, 2,6-dimethyl-[b]	107	C7H9N
1212, 2.370		2-Ethylpyridine[a]	106	C7H9N
1230, 2.500	2 + 3	2,5-Dimethylpyrazine + 2,6-dimethylpyrazine[a]	108	C6H8N2
1248, 2.520		Ethylpyrazine[a,d]	107	C6H8N2
1248, 4.160		2-Ethyl-1H-pyrrole[b]	80	C6H9N
1260, 2.560		2,3-Dimethylpyrazine[a,d]	67	C6H8N2
1308, 2.920		Ethenylpyrazine[a]	106	C6H6N2
1338, 2.260		1*H*-Pyrrole, 1-butyl-[b]	80	C8H13N
1386, 3.610		2-Pyridinecarboxaldehyde[b]	79	C6H5NO
1410, 2.630		3-Ethylpyridine[a]	92	C7H9N
1518, 2.530	4 (5)	2-Ethyl-6-methyl-pyrazine[a]	121	C7H10N2
1530, 2.560	4 (5)	2-Ethyl-5-methyl-pyrazine[a]	121	C7H10N2
1536, 2.590	6	2,3,5-Trimethylpyrazine[a]	42	C7H10N2
1548, 2.580		2-Ethyl-3-methylpyrazine[a,d]	121	C7H10N2
1566, 2.640	7	2-(*n*-Propyl)-pyrazine[a]	94	C7H10N2
1584, 5.270		1*H*-pyrrole-2-carboxaldehyde[a]	66	C5H5NO
1596, 3.820		2-Carboxaldehyde, 1-methyl-1*H*-pyrrole[b]	109	C6H7NO
1602, 2.910		2-Ethenyl-6-methylpyrazine[b]	119	C7H8N2
1620, 2.920		2-Ethenyl-5-methylpyrazine[b,d]	120	C7H8N2
1620, 4.490		4(*H*)-Pyridine, *N*-acetyl-[b]	80	C7H9NO
1638, 3.650		Acetylpyrazine[b,d]	122	C6H6N2O
1686, 3.150		Acetyl pyridine[a,d]	79	C7H7NO
1722, 2.200		1-Pentyl-1*H*-pyrrole[b]	80	C9H15N
1734, 3.260		1-Methyl-2-pyrrolidinone[b]	99	C5H9NO
1764, 5.050		2-Acetylpyrrole[a,d]	94	C6H7NO
1794, 2.180	8 (9)	2-Ethyl-3,5-dimethylpyrazine[a]	135	C8H12N2
1812, 2.170	8 (9)	2-Ethyl-3,6-dimethylpyrazine[a]	135	C8H12N2
1812, 5.500		2-Pyrrolidinone[b]	85	C4H7NO
1818, 2.660		3-Acetyl-1-methylpyrrole[b]	108	C7H9NO
1824, 2.160	11	Tetramethylpyrazine[a]	136	C8H12N2
1866, 2.220		2-Methyl-5-(1-propenyl)-(*E*)-pyrazine[a]	133	C8H10N2
1872, 2.880		1-Pyrrolidinecarboxaldehyde[a]	99	C5H9NO
1920, 2.410		2-Acetyl-3-methylpyrazine[a]	136	C7H8N2O
1932, 2.340		(1-Methylethenyl)-pyrazine[b]	120	C7H8N2O
1932, 2.400		2-Acetyl-6-methylpyrazine[a]	136	C7H8N2O
1944, 2.010		2-Isobutyl-3-methylpyrazine[a,d]	108	C9H14N2
1968, 2.310		1-Propanone, 1-(2-pyridinyl)-[b]	79	C8H9NO
1980, 1.970	12	2,3-Diethyl-5-methyl-pyrazine[a,d]	150	C9H14N2
1986, 1.970		3,5-Diethyl-2-methyl-pyrazine[a,d]	149	C9H14N2
1992, 2.160		5-Methyl-5H-cyclopenta[b]pyrazine[b]	119	C8H10N2
2004, 1.970		Pyrazine, 3,5-diethyl-2-methyl-[b]	150	C9H14N2
2058, 1.990	13	2-Butyl-3-methylpyrazine[a]	108	C9H14N2
2070, 1.920		3,5-Dimethyl-2-isobutyl-pyrazine[a]	122	C10H16N2
2088, 2.570		2-Pyridinecarboxaldehyde[b]	79	C6H5NO
2100, 2.120		2-Methyl-5-(1-propenyl)-pyrazine[b]	134	C8H10N2
2100, 2.280		1-Acetyl-1,2,3,4-tetrahydro-pyridine[b]	82	C7H11NO
2142, 2.120		5,6,7,8-Tetrahydroquinoxaline[b]	134	C8H10N2
2160, 1.940		2-Butyl-3-methylpyrazine[b]	108	C9H14N2

$^{1D}t_R$: first dimension retention time (s); $^{2D}t_R$: second dimension retention time (s).
[a] Compounds identified by both 1D-GC–TOFMS and GC × GC–TOFMS analysis.
[b] Compounds identified only by GC × GC–TOFMS.
[c] Quantification mass (quant mass) used was identical with the unique mass determined automatically by the software as a characteristic mass of the peak.
[d] Compounds reported also in other studies.

In Fig. 7, the elution pattern of volatile chip components is illustrated, with *N*-heterocyclic compounds located in the upper part of the contour plot, above alkanes and aromatic hydrocarbons, aldehydes, and ketones.

Within the group, the position on *y*-axis (second dimension retention time) increased in the order: pyridines < pyrazines < pyrrols; the exception were *N*-substituted pyrrols. These compounds, due to the considerable decreased polarity of their molecules, elute at lower second dimension retention times compared to *C*-substituted pyrrols with the free NH group in the pyrrole ring. Within the group of the substituted pyrazines, the position on *y*-axis increased in the order: alkylpyrazines < alkenylpyrazines < cycloalkylpyrazines < acetylpyrazines.

Only 13 from the 57 compounds (including the target pyrazines) listed in Table 1 were previously reported in earlier studies concerned with volatiles occurring in potato chips [13,14,21,22]. In many cases there was a discrepancy in the substitution pattern tentatively proposed for the particular structural category. Although the orthogonal GC separation employed in our experiments reduced the co-elutions compared to conventional separation car-

Table 2
Performance characteristics of the SPME combined with GC–ITMS and GC × GC–TOFMS techniques.

No.	Pyrazine	GC–ITMS					GC × GC–TOFMS				
		Linear range (μg kg^{-1})	R^2	LOQ (μg kg^{-1})	RSD (%)	Concentration level (μg kg^{-1})	Linear range (μg kg^{-1})	R^2	LOQ (μg kg^{-1})	RSD (%)	Concentration level (μg kg^{-1})
1	Methylpyrazine	75–6400	0.9989	64	9	369	4–650	0.9995	3	4	250
2	2,5-Dimethylpyrazine[a]	140–7200	0.9990	121	6	460	8–2050	0.9957	6	4	430
3	2,6-Dimethylpyrazine										
4	2-Ethyl-5(6)-methylpyrazine	65–3000	0.9980	63	5	<LOQ	8–840	0.9964	8	7	30
5	2-Ethyl-5(6)-methylpyrazine[a]	60–3000	1.0000	46	5	174	12–660	0.9951	8	7	69
6	2,3,5-Trimethylpyrazine										
7	2-*n*-Propylpyrazine	65–3400	0.9992	44	7	73	8–630	0.9979	8	15	8
8	2-Ethyl-3,5(3,6)-dimethylpyrazine	60–4000	0.9986	48	8	142	8–600	0.9982	8	8	36
9	2-Ethyl-3,5(3,6)-dimethylpyrazine[a]	130–8800	0.9987	83	–	<LOQ	12–1200	0.9956	8	16	17
10	2,3-Diethylpyrazine										
11	2,3,5,6-Tetramethylpyrazine	65–3500	0.9945	62	–	<LOQ	4–370	0.9939	3	17	9
12	2,3-Diethyl-5-methylpyrazine	50–3300	0.9954	51	–	<LOQ	4–330	0.9993	3	18	3
13	2-Butyl-3-methylpyrazine	65–4000	0.9958	63	–	<LOQ	4–630	0.9900	3	16	4

Note: For SPME–GC–ITMS analysis, commercially prepared samples and for SPME–GC × GC–TOFMS lab-fried ones were used for the experiment.
[a] Only common characteristics are available because of co-elution.

Fig. 7. GC × GC–TOFMS chromatogram of potato chips sample with denotation of structural groups separation.

ried out by other authors, we were not able to support the isomers identification by using the retention indexes since they are not available for the stationary phase used in our study.

3.5. Performance characteristics

As a part of the method validation, the stability of sample composition during sample storage in a refrigerator was tested. No significant changes in aroma profile of chips were found after 1 month of storage.

Repeatability of both SPME–GC–ITMS and SPME–GC × GC–TOFMS methods was characterised as a relative standard deviation (RSD), calculated for each analyte from six repetitive analyses of a deep fried (20 min/160 °C) potato chips sample; the values for individual analytes are shown in Table 2. The levels of target pyrazines were determined by the external calibration method using the matrix-matched standards (see Section 2).

Limits of quantification (LOQs) were estimated by the analyses of the lowest calibration (matrix-matched) standard. The LOQs were estimated as the level with S/N ratio >10.

In spite of the shorter extraction time (15 min *vs.* 60 min), the LOQs of target analytes were approximately 10 times lower in GC × GC–TOFMS system as compared to GC–ITMS. On the other hand, GC–ITMS enabled measurements at higher levels of pyrazines (for comparison see the linear ranges in Table 2). Nevertheless, the linear range of the SPME–GC × GC–TOFMS method, even if narrower than the range of the SPME–GC–ITMS method, is still sufficient for measuring the real levels of pyrazines occurring in potato chips samples.

The calibration standard mixtures of pyrazines at six concentration levels in methanol were prepared for the method linearity testing. Matrix-matched calibration was used for the quantification of pyrazines for which the standards were commercially available. As the most similar matrix, but free of the target compounds (blank sample), pre-fried potato chips (5 min/160 °C) were employed. Five microliters of each calibration standard was added to the 1.5 g of "blank" matrix. The linearity and the regression coefficients of individual analytes are summarised in Table 2. It was found that GC × GC–TOFMS is more sensitive technique; thus, the lowest calibration level was approximately 10 times lower than for GC–ITMS. The linear range was in both cases two orders of magnitude and the regression coefficients (R^2) higher than 0.995.

4. Conclusions

A simple head-space SPME method can be used for extraction of pyrazines and other volatile compounds from potato chips head-

space. A brief assessment of the results obtained in three alternative GC–MS set-ups employed for analyses (HP-VOC column used in all experiments) of volatiles thermally desorbed from (30/50 μm DVB/CAR/PDMS) SPME fibre in GC injection port can be summarized as follows:

(i) GC–ITMS—chromatographic resolution of three isomeric critical pairs of pyrazines was not possible under given experimental conditions; moreover, the limits of quantification of other target pyrazines were, due to a relatively low sensitivity of the particular mass analyser, relatively high.
(ii) 1D-GC–TOFMS—thanks to the spectral deconvolution enabled by TOF mass analyser, only one of the three co-eluting isomeric pyrazine pairs remained unresolved. Compared to GC–ITMS, some improvement of pyrazines LOQs occurred.
(iii) GC × GC–TOFMS—separation of the three critical pairs of pyrazines was (rather surprisingly) not achieved, probably due to the recombination of target analytes during the modulation process. On the other hand, better signal-to-noise ratio for target compounds was obtained; their LOQs were reduced compared to one-dimensional system although the SPME sorption time was reduced by factor of ≈4. The separation of other volatile sample components was fairly better, what enabled non-target screening of volatiles profile and tentative identification (based on the NIST library search) some of them. In addition to the target pyrazines, other 46 nitrogen-containing heterocyclic compounds (pyrazines, pyrrols, pyridines, pyrrolidinones, and tetrahydropyridines) exceeded the established identification criteria (spectral match >700).

The follow-up study will be focused on identification of the volatile Maillard products, release of which correlates with acrylamide formation during potato chips frying, and which could enable an easy on-line control of this processing contaminant.

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References

[1] K.G. Lee, T. Shibamoto, Food Rev. Int. 18 (2002) 151.
[2] R.G. Buttery, D.G. Guadagni, L.C. Ling, J. Sci. Food Agric. 24 (1973) 1125.
[3] E.C. Coleman, C.-T. Ho, S.S. Chang, J. Agric. Food Chem. 29 (1981) 42.
[4] H.E. Nursten, M.R. Sheen, J. Sci. Food Agric. 25 (1974) 643.
[5] D.B. Josephson, R.C. Lindsay, J. Food Sci. 52 (1987) 328.
[6] M. Zviely, E.A. Hodrien, Perfum. Flavorist 28 (2003) 32.
[7] IARC, Some Industrial Chemicals, International Agency for Research on Cancer, Lyon, France, 1994.
[8] E. Tareke, P. Rydber, P. Karlsson, S. Eriksson, M. Törnqvist, J. Agric. Food Chem. 50 (2002) 4998.
[9] A. Becalski, B.P.-Y. Laud, D. Lewis, S.W. Seaman, J. Agric. Food Chem. 51 (2003) 802.
[10] D. Taeymans, J. Wood, P. Ashby, I. Blank, A. Studer, R.H. Stadler, P. Gondé, P.V. Eijck, S. Lalljie, H. Limgnert, M. Linblom, R. Matissek, D. Muller, D. Tallmadge, J. O'Brien, S. Thompson, D. Silvani, T. Whitmore, Crit. Rev. Food Sci. Nutr. 44 (2004) 323.
[11] Y. Zhang, G. Zhang, Y. Zhang, Review and recent developments, J. Chromatogr. A 1075 (2005) 1–21.
[12] S. Ehling, T. Shibamoto, J. Agric. Food Chem. 53 (2005) 4813.
[13] R.G. Buttery, R.M. Seifert, D.G. Guadagni, L.C. Ling, J. Agric. Food Chem. 19 (1971) 969.
[14] M.J. Oruna-Concha, S.C. Duckham, J.M. Ames, J. Agric. Food Chem. 49 (2001) 2414.
[15] R. Wagner, W. Grosch, Lebens. -Wiss. -Technol. 30 (1997) 164.
[16] M.A. Majcher, H.H. Jeleń, J. Agric. Food Chem. 53 (2005) 6432.
[17] H. Kataoka, H.L. Lord, J. Pawlisyzn, J. Chromatogr. A 880 (2000) 35.
[18] T. Cajka, J. Hajslova, LC GC Eur. 20 (1) (2007) 25.
[19] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[20] T. Cajka, J. Hajslova, J. Cochran, K. Holadova, E. Klimankova, J. Sep. Sci. 30 (2007) 534.
[21] S.C. Duckham, A.T. Dodson, J. Akker, J.M. Ames, Nahrung/Food 45 (2001) 317.
[22] B. Sigmund, M. Murkovic, Food Chem. 84 (2004) 367.